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ORIGINAL PAPER

Inactivation of phosphomannose isomerase gene abolishes sporulation and antibiotic production in *Streptomyces coelicolor*

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Abstract Phosphomannose isomerases (PMIs) in bacteria and fungi catalyze the reversible conversion of D-fructose-6-phosphate to D-mannose-6-phosphate during biosynthesis of GDP-mannose, which is the main intermediate in the mannosylation of important cell wall components, glycoproteins, and certain glycolipids. In the present study, the kinetic parameters of PMI from *Streptomyces coelicolor* were obtained, and its function on antibiotic production and sporulation was studied. *manA* (SCO3025) encoding PMI in *S. coelicolor* was deleted by insertional inactivation. Its mutant (*S. coelicolor*Δ*manA*) was found to exhibit a *bld*-like phenotype. Additionally, *S. coelicolor*Δ*manA* failed to produce the antibiotics actinorhodin and red tripyrolle undecylprodigiosin in liquid media. To identify the function of *manA*, the gene was cloned and expressed in *Escherichia coli* BL21 (DE3). The purified recombinant ManA exhibited PMI activity (K_{cat}/K_m (mM^{-1} s^{-1}=0.41 for D-mannose-6-phosphate), but failed to show GDP-D-mannose pyrophosphorylase [GMP (ManC)] activity. Complementation analysis with *manA* from *S. coelicolor* or *E. coli* resulted in the recovery of *bld*-like phenotype of *S. coelicolor*Δ*manA*. SCO3026, another ORF that encodes a protein with sequence similarity towards bifunctional PMI and GMP, was also tested for its ability to function as an alternate ManA. However, the purified protein of SCO3026 failed to exhibit both PMI and GMP activity. The present study shows that enzymes involved in carbohydrate metabolism could control cellular differentiation as well as the production of secondary metabolites.

Keywords Phosphomannose isomerase · Sporulation · Pigment production · Complementation analysis · Antibiotic production

Thangamani Rajesh and Eunjung Song contributed equally to this work.

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Introduction

Phosphomannose isomerases (PMIs) catalyze the reversible conversion of D-fructose-6-phosphate to D-mannose-6-phosphate, which is subsequently acted upon by phosphomannomutase (PMM) and GDP-D-mannose pyrophosphorylase (GMP) to generate mannosylated glycans. These products constitute important cell wall components in bacteria and fungi, nucleotide sugars, glycoproteins, and certain glycolipids (Dunwell et al. 2000; Sousa et al. 2010). PMIs in

general are classified into four structurally unrelated distinct classes: type I PMIs that require zinc as a cofactor for their enzymatic activity, type II PMIs that exhibit both PMI and GMP activities, type IV with both PMI and phosphoglucoisomerase activities, and type III with a single protein from *Sinorhizobium meliloti* (Hansen et al. 2004; Jensen and Reeves 1998). PMIs have been extensively studied in bacteria and are important in controlling a wide variety of functions. Type I PMIs that catalyze a single isomerization reaction have been described in bacteria, fungi, and eukaryotes. The crystal structure of a single type I PMI from *Candida albicans* providing insights into the active site and metal ion-binding site has also been reported (Cleasby et al. 1996).

Based on functional studies, it has been proposed that type I PMIs essentially control the survival, differentiation, and pathogenesis of an individual organism. A prominent role for type I PMIs in cell growth with mannose as the sole carbon source has been identified in *Escherichia coli*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Saccharomyces cerevisiae*, and *Aspergillus fumigatus* (Elbaz and Ben-Yehuda 2010; Fang et al. 2009; Pitkänen et al. 2004; Sampaio et al. 2003; Sasaki et al. 2011). In *B. subtilis*, PMI not only controls cellular growth but also cell wall integrity and chromosome morphology (Elbaz and Ben-Yehuda 2010). Similarly, in *A. fumigatus*, PMI has been shown to be critical for cellular survival, as its deletion leads to defects in cell wall biosynthesis (Fang et al. 2009). Recently, PMI from *Photorhabdus luminescens* was shown to influence exopolysaccharide production and maintenance, thereby controlling biofilm formation (Amos et al. 2011). Although these studies emphasize the essential roles of PMIs, only limited information is available concerning their effects on the regulation of secondary metabolite production (Lochlainn and Caffrey 2009).

Streptomyces coelicolor is the most well-studied *Streptomyces* strain and produces two major secondary metabolites, the pigmented antibiotics red tripyrolle undecylprodigiosin (RED) and blue polyketide actinorhodin (ACT) during the transition from vegetative growth to aerial growth (Baltz 1998; Lee et al. 2006). Antibiotic production and sporulation in *S. coelicolor* are highly affected by the carbon source that includes glucose, xylose, maltose, and *N*-acetylglucosamine. We previously studied these processes utilizing mannose. We showed that ManB has both PMM and phosphoglucomutase activities, and deletion of *manB* results in a dramatic increase in ACT production in low-glucose Difco nutrient medium (Yang et al. 2010). As a sequential work, we studied the role of ManA in antibiotic production. We described a novel role for *manA*, in which it encodes a PMI that affects both sporulation and antibiotic production in *S. coelicolor*. These results indicate that carbohydrate metabolic pathway enzymes could also be involved in controlling the cellular development and production of secondary metabolites in *S. coelicolor*.

Materials and methods

Bacterial strains and media

Bacterial strains, primers, and plasmids used in this study are listed in Table 1. *S. coelicolor* A3(2) M145 was obtained from the Korean Collection for Type Cultures (KCTC, South Korea). *E. coli* DH5α and *E. coli* BL21 (DE3) used as host strains for transformation and protein overexpression, respectively, were from the laboratory stock. *S. coelicolor* A3 (2) M145 was grown in R5$^-$ medium composed of 103 g of sucrose; 0.25 g of K_2SO_4; 10.12 g of $MgCl_2{\cdot}6H_2O$; 10 g of glucose; 0.1 g of Difco casamino acids; 2 mL of trace element solution comprised of $ZnCl_2$ 40 mg, $FeCl_3{\cdot}6H_2O$ 200 mg, $CuCl_2{\cdot}2H_2O$ 10 mg, $MnCl_2{\cdot}4H_2O$ 10 mg, $Na_2B_4O_7{\cdot}10H_2O$ 10 mg, and $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 10 mg in 1 L of deionized water; 5 g of yeast extract; 5.73 g of TES buffer; and 7 mL of 1 N NaOH in 1 L of distilled water (Kieser et al. 2000). R5$^-$-fructose and R5$^-$-mannose were made by replacing 10 g of glucose with 10 g of fructose and 10 g of mannose. The liquid cultures were grown at 30°C with shaking at 200 rpm in a Hanbaek shaker for 5 days (Hanbaek Scientific Co, South Korea). *E. coli* strains were routinely cultured in Luria–Bertani (LB) agar and/or liquid broth composed of 3 g of beef extract, 5 g of peptone, and 15 g of agar in 1 L of distilled water. For expression analysis, *E. coli* cultures were grown at 37°C with shaking at 220 rpm. Antibiotics such as thiostrepton (30 μg/mL) dissolved in DMSO for transformation of *S. coelicolor*, 50 μg/mL of kanamycin, and 100 μg/mL of ampicillin for transformation of *E. coli* were added when required to select transformants and maintain plasmids.

DNA manipulations

Cultivation of *S. coelicolor* strains was performed by following standard procedures (Kieser et al. 2000). Fresh spores of *S. coelicolor* were collected from R5$^-$ agar plates. The entire *manA* (SCO3025) and SCO3026 were obtained by polymerase chain reaction (PCR) using the primer pair 28manA F–28manA R/28manA2 F–28manA2 R and chromosomal DNA as a template, followed by cloning at the *Nde*I and *Hin*dIII sites of plasmid pET28ma. The resulting recombinant plasmids (p28manA1 and p28-3026) were transformed into *E. coli* BL21 (DE3). For complementation analysis, *manA* gene sequences from *E. coli* and *S. coelicolor* were amplified using the primer pair SCmanA F–SCmanA R/ECmanA F–ECmanA R, followed by cloning at the *Eco*RI and *Hin*dIII sites of pIBR25 to produce recombinant plasmids p25ECMA and p25SCMA, respectively. The recombinant plasmids were initially transformed into *E. coli* JM110, re-isolated, and then used to transform *S. coelicolor*Δ*manA*. SCO3025 encoding ManA was deleted and replaced with an

Table 1 List of bacterial strains, primers, and plasmids used in this study

Strain/primer/plasmid	Relevant information	Source/reference
Bacterial strains		
E. coli strains		
DH5α	F$^-$ϕ80*lacZ* M15 *endA recA hsdR*($r_k^-m_k^-$) *supE thi gyrA relA* Δ(*lacZYA-argF*)U169	Laboratory stock
BL21 (DE3)	F$^-$*ompT hsdS$_B$*($r_B^-m_B^-$) *gal dcm*	Novagen
JM110	*dam*$^-$, *dcm*$^-$	Laboratory stock
S. coelicolor A3(2) M145	SCP1$^-$, SCB2$^-$, Pgl$^+$	KCTC
*S. coelicolor*Δ*manA*	*manA*-deleted mutant	This study
*S. coelicolor*Δ*manA*/p25ECMA	*S. coelicolor*ΔmanA carriying p25ECMA	This study
*S. coelicolor*Δ*manA*/p25SCMA	*S. coelicolor*ΔmanA carriying p25SCMA	This study
Primers		
28manA F	5′-CGCGCATATGGACCGCCTCGACAACAC-3′	This study
28manA R	5′-TATAAAGCTTTCAGACGCGCACGGTGGCC-3′	This study
28manA2 F	5′-GCCG GAATTCCATATGCTCGACGAATCGCTCCTCGACG-3′	This study
28manA2 R	5′-GCCGAAGCTTTCAGGCTCCCGAGGCGAGCG-3′	This study
SC manA F	5′-GCCGGAATTCCATATGGACCGCCTCGACAACAC-3′	This study
SC manA R	5′-GCCGAAGCTTTCAGACGCGCACGGTGGCC-3′	This study
EC manA F	5′-GCCGGAATTCCATATG CAAAAACTCATTAACTCAGTGC-3′	This study
EC manA R	5′-GCCGAAGCTTTTACAGCTTGTTGTAAACACGCGC-3′	This study
Plasmids		
pET24ma	p15A replication origin, T7 lac promoter, C-terminal his-tag coding, kan^R	Yang et al. (2005)
pIBR25	pWHM3 carrying *ermE** promoter from *Saccharopolysopra erythraea*	Sthapit et al. (2004)
p28manA1	pET28ma carrying PCR product of *manA* from *S. coelicolor*	This study
p28-3026	pET28ma carrying PCR product of SCO3026 from *S. coelicolor*	This study
p25ECMA	pIBR25 carrying PCR product of *manA* from *S. coelicolor*	This study
p25SCMA	pIBR25 carrying PCR product of *manA* from *E. coli*	This study

apramycin resistance gene by PCR targeting using oligonucleotide primers with 5′ ends overlapping the upstream (36 bp) and downstream (36 bp) regions of the SCO3025 coding sequence and 3′ (priming) ends designed to amplify the apramycin resistance disruption cassette of pIJ773 and the corresponding cosmid. The mutant was constructed as described previously (Bierman et al. 1992). Plasmid DNA preparation, restriction enzyme digestion, ligation, and transformation of *E. coli* were performed essentially as reported (Sambrook et al. 1998).

Expression and purification of recombinant ManA and SCO3026

Plasmids p28manA1 or p28-3026 were transformed into *E. coli* BL21 (DE3), and the resulting transformants were grown at 37 °C with shaking at 220 rpm in 100 mL of LB broth containing 50 mg/L of kanamycin. The cells were grown to an OD of 0.6 at 600 nm, after which 1 mM IPTG was added to the cell broth to induce recombinant protein expression. The cells were harvested after 12 h of induction at 30 °C. For enzyme preparations, the harvested cells were washed and then suspended in 5 mL of 50 mM Tris–HCl buffer (pH 7.5) containing 0.2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.01% (*v*/*v*) 2-mercaptoethanol, and 1 mM 1,4-dithiothreitol. Following this, cells were subjected to ultrasonication for 5 min with ten cycles consisting 15 s each with an interval of 15 s at 4°C (Vibra Cell™ Sonics Scientific, Inc). The supernatant solution containing the soluble proteins was prepared by centrifugation (17,000×*g*, 20 min). For purification of His-tagged proteins, soluble proteins were applied to Ni^{2+}-NTA beads pre-equilibrated with base buffer (50 mM NaH_2PO_4, 0.05% NaCl, 0.05% Tween 20, pH 8.0). Following a 2-h binding reaction, unbound proteins were removed by washing four times with the same buffer containing 20 mM imidazole. Both purified enzymes were eluted three times from the column with

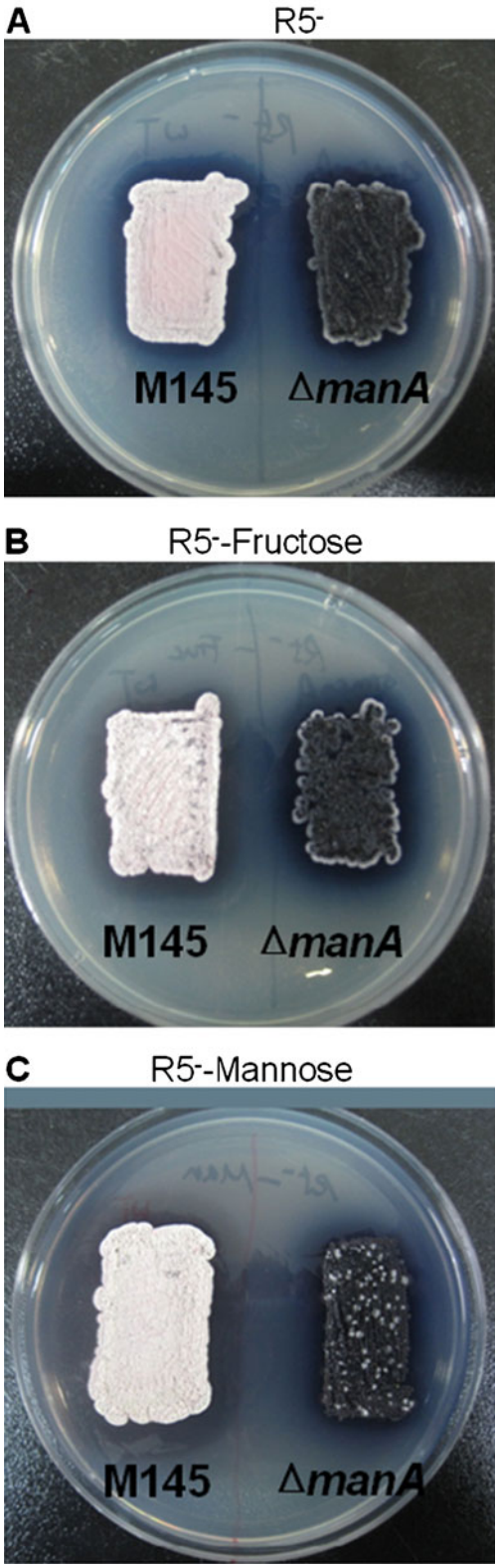

Fig. 1 Phenotypes of the parent strain of *S. coelicolor* and its mutant in solid R5⁻ media with different carbon sources such as glucose (**a**), fructose (**b**), and mannose (**c**). Strains were grown on R5⁻ plate with respective carbon sources for 6 days. The parent strain of *S. coelicolor* sporulated abundantly, and *manA*-deleted mutant failed to sporulate and exhibited a sporulation-null phenotype

250 mM imidazole buffer, and each purification step was individually analyzed by 12% SDS-PAGE.

Determination of kinetic parameters for ManA

The protein concentration of His-tag-purified ManA was measured with a Bradford assay kit (Bio-Rad Co., USA). PMI activity was determined by following slightly modified previous methods (McCarthy et al. 2005). For PMI activity, 5 μL of purified enzyme (0.37 μg/μL) was mixed with 25 μL of phosphate buffer (0.1 M, pH 7.5), 2 μL of $MgCl_2$ (1 M), 0.5 μL of glucose-6-phosphate dehydrogenase (Roche, Germany), phosphoglucose isomerase (Roche, Germany), 2 μL of $NADP^+$ (50 mM), 55.4 μL of distilled water, and 10 μL of mannose-6-phosphate (100 mM), followed by incubation at 30 °C for 5 min. The rate of change in absorbance was measured in 100 μL of each sample at 340 nm using a 96-well plate and multiscanner (Thermo Electron Corp., Finland). For GMP activity, 50 mM Tris–HCl (pH 7.5), 5 mM Man1-P, 5 mM $MnSO_4$ ($MgCl_2$), 5 mM GTP, and 10 μL of Enz were mixed in a 50-μL reaction at 30 °C for 30 and 60 min. Thin layer chromatography (TLC) was then performed with TLC eluent (isopropanol/25% ammonium hydroxide/water= 10:5:1). The spots were developed using 10% H_2SO_4 in ethanol and heated.

Complementation of *S. coelicolor*Δ*manA*

Plasmids p25ECMA and p25SCMA were transformed into *S. coelicolor*Δ*manA* by a protoplast-mediated method. Briefly, *S. coelicolor*Δ*manA* was cultured in 25 mL of YEME medium for 48 h at 30°C with shaking at 200 rpm, followed by cell harvesting by centrifugation at 1,000×*g* for 10 min. The cell pellet was then resuspended in 15 mL of 10.3% sucrose and centrifuged as above. This washing step was repeated twice. After washing, the mycelium was resuspended in 4 mL of P buffer (Kieser et al. 2000) containing 1 mg/mL of lysozyme. The tubes were incubated at 30°C for 45 min, after which 6 mL of P buffer was added. The prepared protoplasts were filtered using cotton wool, transferred to 15 mL centrifuge tubes, and centrifuged at 3,000×g for 10 min. Then, the supernatant was discarded and the protoplasts in the pellet fraction resuspended in 50 mL of P buffer. To the prepared protoplasts, 12 μL of individual plasmids was added, followed by immediate addition of 200 μL of T buffer (Kieser et al. 2000) and 500 μL of P buffer. The contents in the tubes were mixed gently, plated on R5⁻ agar medium, and incubated overnight at 30°C. The next day, the plates were overlayed with 1 mL of P buffer containing thiostrepton. Positive transformants were segregated twice and used for further analysis.

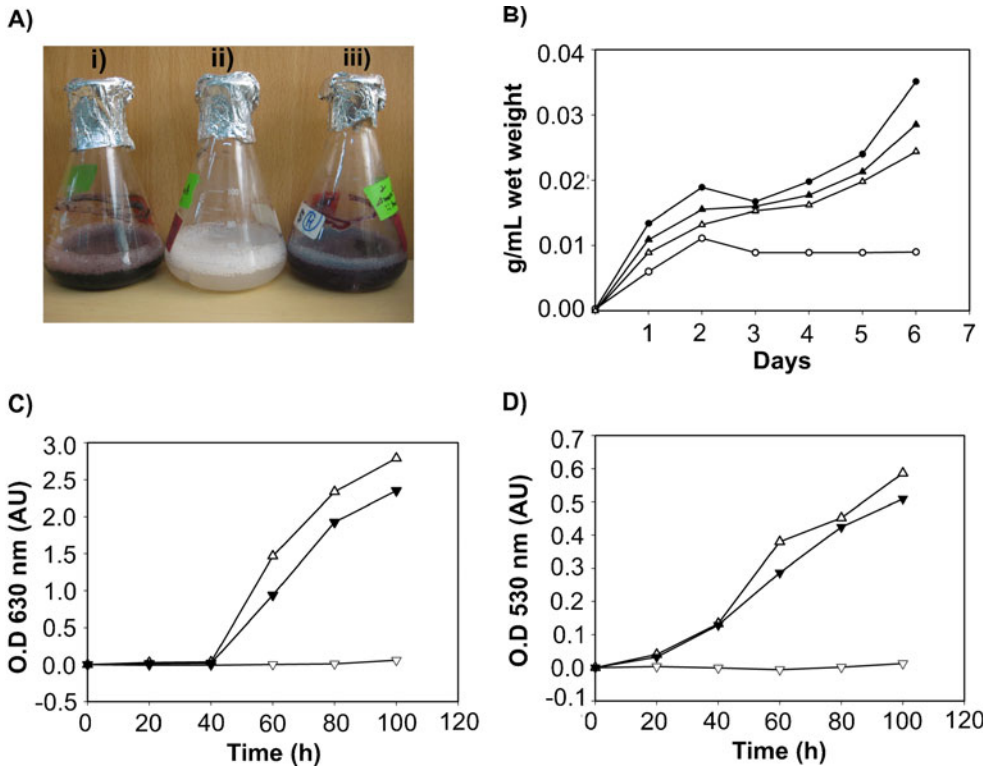

Fig. 2 Antibiotic production in *S. coelicolor* and its mutants in liquid media. **a** The parent strain of *S. coelicolor* produced both the pigmented antibiotics ACT and RED (*i*). *S. coelicolor*Δ*manA* was found to exhibit a null phenotype for antibiotic production in liquid media (*ii*). However, antibiotic production was recovered upon complementation of *S. coelicolor*Δ*manA* with *manA* (*iii*). **b** Growth of *S. coelicolor* parent strain (M145) (*filled circle*), *S. coelicolor*Δ*manA* (*empty circle*), *S. coelicolor*ΔmanA/p25ECMA (*filled triangle*), and *S. coelicolor*Δ*manA*/p25SCMA (*empty triangle*) are shown. ACT (**c**) and RED (**d**) production in *S. coelicolor* parent strain (M145)

Scanning electron microscopy

The spores and hyphae of all the samples were observed by field emission scanning electron microscopy (FE-SEM) (AURIGA, Germany) after 5 days of growth on R5⁻ media. The cut agar blocks were pre-fixed with 2% paraformaldehyde and 2% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) and then post-fixed with 1% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH 7.2) for 2 h, followed by dehydration and drying. Each sample was platinum sputter-coated and examined by FE-SEM.

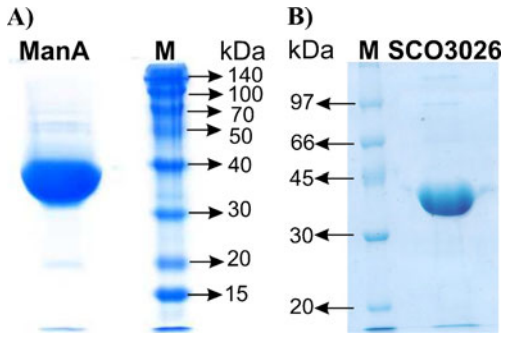

Fig. 3 SDS-PAGE analysis of purified ManA (**a**) and SCO3026 (**b**). His tag-purified ManA (40 kDa) and SCO3026 of (42 kDa) are shown. *Lane M*: molecular weight protein marker

Antibiotic production assays

Antibiotic production was examined in liquid and solid cultures. For the liquid cultures, cells from the R5⁻ solid plates were inoculated and grown for 24 h. Then, they were washed twice with sterilized water and diluted to have the same wet weight in the same amount of water. Exactly 20 mg (wet weight) of cells was inoculated and cultured in a 250-mL baffled flask with 50 mL of R5⁻ without additional carbon sources. The cultures were grown at 30°C with shaking at 200 rpm. One milliliter of each sample was obtained at the specified times. After centrifugation in a micro-centrifuge for 10 min, 900 μL of the supernatant was sampled for further analysis according to previously reported procedures (Bystrykh et al. 1996). ACT and RED were determined spectrophotometrically at 633 and 530 nm, respectively, in the treated samples (150 μL) using a 96-well plate and multiscanner (Thermo Electron Corp., Finland).

Table 2 Kinetic values of various PMIs

S. no	Organism	Parameter		Reference
		K_m (mM)	K_{cat}/K_m (mM^{-1} s^{-1})	
1	*P. aeruginosa* PAO1	3.03	ND	Shinabarger et al. (1991)
2	*Burkholderia cepacia*	9.01	2.1	Sousa et al. (2007)
3	*Xanthomonas campestris*	2.0	ND	Papoutsopoulou and Kyriakidis (1997)
5	*H. pylori* 26695	0.055	0.00011	Wu et al. (2002)
6	*E. coli*	0.33	70	Roux et al. (2011)
7	*S. coelicolor*	0.90	0.41	This study
8	*C. albicans*	1.24	ND	Coulin et al. (1993)
9	*S. cerevisiae*	0.65	ND	Wells et al. (1993)
10	*A. fumigatus*	2.59	9.7	Fang et al. (2009)
11	*Homo sapiens*	0.043	840	Roux et al. (2011)

Results

Deletion of *manA* results in *bld*-like phenotype

The main objective of the present study was to identify the functional role of *manA* (SCO3025) in *S. coelicolor*. Initially, the *manA* gene was inactivated by replacing it with apramycin resistance gene. The *manA*-deleted strain, *S. coelicolor*Δ*manA*, exhibited a *bld*-like phenotype in solid medium (Fig. 1a). When streaked on a $R5^-$ plate, *S. coelicolor*Δ*manA* grew slowly and failed to produce spores. Spores could not be collected by a known spore preparation method (Kieser et al. 2000) and it is different from *manB* mutant, which showed delayed aerial mycelium formation and sporulation. When glucose was changed to fructose or mannose, the mutant retained its sporulation-defective phenotype, similar to *bld* mutant (Fig. 1b, c) (Kelemen et al. 2001). Partial spore production with mannose-supplemented complex media ($R5^-$-mannose) was observed; however, the spores were not well defined and displayed a different shape from M145 strain by normal microscopic analysis and SEM, suggesting the sporulation system was still not working properly (Fig. 1c).

Deletion of *manA* abolishes antibiotic production in complex media

Based on the sporulation results, the pattern of antibiotic production was also examined. Although *S. coelicolor*Δ*manA* showed a deficiency in sporulation when cultured on the solid plate, the level of pigment production was higher in comparison to its parent strain. In liquid cultures, *S.*

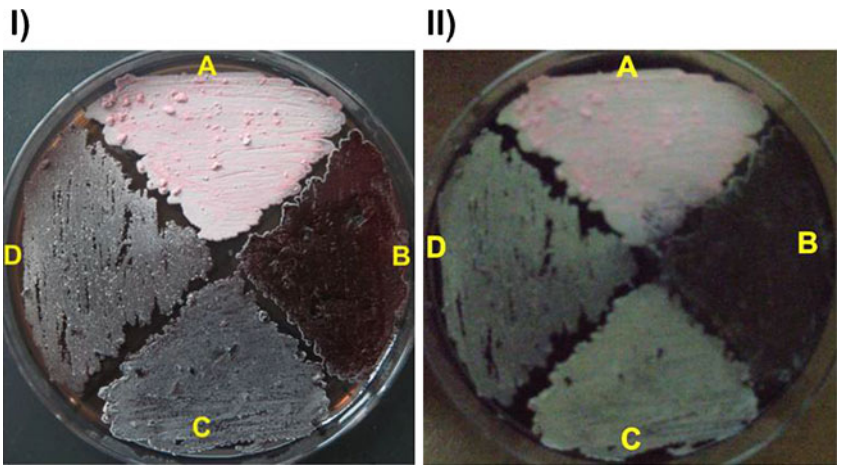

Fig. 4 Phenotypes of *S. coelicolor* and various complemented mutants in solid media. Strains were grown on $R5^-$ plate with mannose as carbon source for 6 days. Sporulative phenotype at day 4 (*I*) and day 6 (*II*) of parent *S. coelicolor* (*A*), *bld*-like phenotype of *manA*-deleted mutant (*B*), and recovery from sporulation-defective phenotype by complementation with *S. coelicolor*Δ*manA*/p25SCMA (*C*) and *S. coelicolor*Δ*manA*/p25ECMA (*D*) are shown

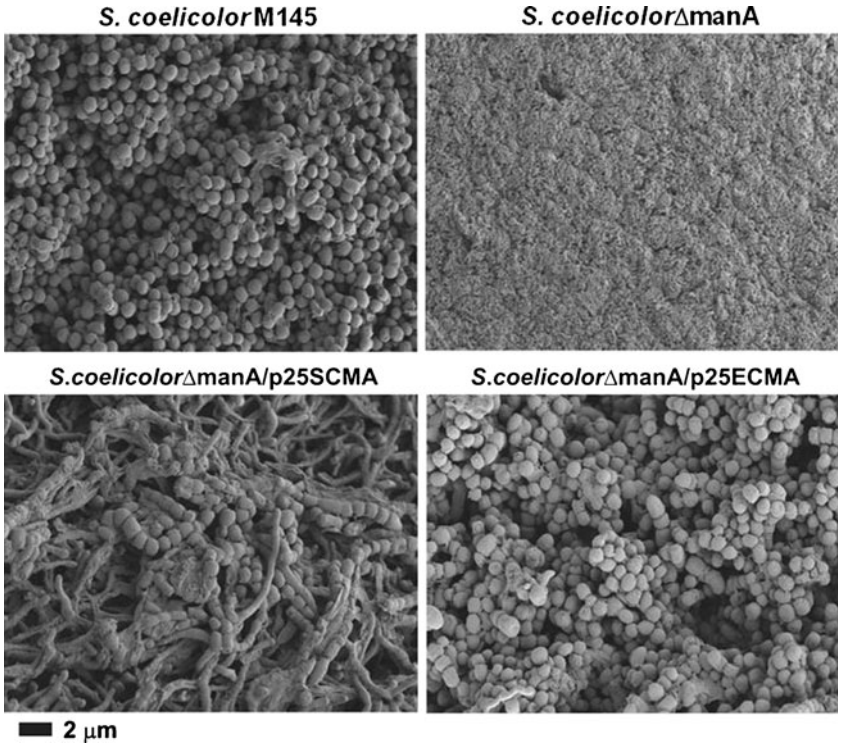

Fig. 5 Phenotypes of *S. coelicolor* and various complemented mutants as observed by FE-SEM. Samples were prepared with strains shown in Fig. 4 on the sixth day. Distinct sporulation was observed with the parent strain (*S. coelicolor* M145), whereas *manA* mutant failed to produce viable spores (*S. coelicolorΔmanA*). Partial recovery from sporulative phenotype was observed with *manA* from *S. coelicolor* (*S. coelicolorΔmanA*/p25SCMA), and 100% recovery of sporulative phenotype similar to parent strain was observed with *manA* from *E. coli* (*S. coelicolorΔmanA*/p25ECMA)

coelicolorΔmanA failed to produce the antibiotics ACT and RED, but successful complementation occurred with *manA* gene from *S. coelicolor* (Fig. 2a). For the liquid cultures, cells from the $R5^{-}$ solid plates were inoculated in 3 mL for 24 h and washed twice with sterilized water. Then, the cells were diluted to have the same wet weight and inoculated to 25 mL. The growth of *S. coelicolorΔmanA* was slow, and antibiotic production was abolished in liquid culture (Fig. 2b–d). Interestingly, when *S. coelicolorΔmanA* from the solid plate was inoculated and grown for 3 days without washing, it produced ACT; however, when grown and washed with sterilized water after 1 day, it could not produce any antibiotics, suggesting that there were some other metabolites or factors that affected antibiotic production in liquid culture. These results indicate that *manA* from *S. coelicolor* encodes a vital enzyme essential to antibiotic production in liquid culture.

SCO3025 encodes PMI (ManA)

To identify the function of *manA* in *S. coelicolor*, the entire *manA* gene was cloned and overexpressed in *E. coli* BL21 (DE3). Recombinant ManA was purified to homogeneity (Fig. 3a) and assayed for PMI and GMP activities. ManA exhibited PMI activity with a substrate specificity of K_m 0.90 mM towards D-mannose-6-phosphate. The obtained K_{cat}/K_m value for ManA was 0.41 mM^{-1} s^{-1}. A comparison between reported K_m values for different organisms and the K_{cat}/K_m value of *E. coli* (70 mM^{-1} s^{-1}) indicated that this enzyme had low enzymatic activity (Table 2). ManA protein could use Mg^{2+} ions for its activity despite the presence of a conserved Zn-binding motif as reported previously (Jensen and Reeves 1998). The order of metal ion requirement for ManA was Mg^{2+}>Cu^{2+}>Zn^{2+} due to which the activity levels were 100%, 90%, and 70%, respectively. However, the protein failed to exhibit GMP activity when tested by TLC analysis (data not shown). SCO3026, another ORF in *S. coelicolor*, was found to have sequence similarity with proteins that could have both PMI and GMP activities (Supplementary material S1). Thus, this protein was also cloned, overexpressed in *E. coli* BL21 (DE3), and analyzed for PMI and GMP activities. The purified recombinant protein of SCO3026 (Fig. 3b) failed to exhibit both PMI and GMP activities.

Complementation of *S. coelicolorΔmanA*

To confirm that ManA truly influences sporulation, complementation analyses were performed with *manA* from *E. coli* and *S. coelicolor*. *S. coelicolorΔmanA*/pECMA and *S. coelicolorΔmanA*/pSCMA both retained the ability to produce visible spores in solid medium (Fig. 4). Scanning electron microscopic analysis revealed that *manA* from *S. coelicolor* was able to recover from the *bld*-like phenotype only to a certain extent, as viable spores and defective hyphae were observed with similar frequency (Fig. 5). Complementation of *S. coelicolorΔmanA* with *manA* from *E. coli* resulted in higher recovery from the *bld*-like

phenotype (Fig. 5). The observed difference in complementation analysis could be attributed to differences in the activities of the individual enzymes based on their kinetic parameters. However, in *S. coelicolor*Δ*manA*/pSCMA, the recovery of antibiotic production was comparable to that of the parent strain (Fig. 2c, d).

Discussion

ManA, which catalyzes the isomerization of fructose-6-phosphate to mannose-6-phosphate, is a vital enzyme in the biosynthesis of nucleotide sugars, including GDP-fructose and UDP-GlcNAc (van Heijenoort 2007; Wu et al. 2002). Both of these sugars are involved in peptidoglycan and teichoic acid biosynthesis. In most cases, a defect in ManA production leads to defects in cell wall arrangement. For instance, capsular biosynthesis in *Helicobacter pylori*, cell wall synthesis and morphogenesis in *A. fumigatus*, and cell wall integrity in *B. subtilis* have all been shown to be aberrant when *manA* expression is altered (Elbaz and Ben-Yehuda 2010; Fang et al. 2009; Wu et al. 2002). Although PMIs have been extensively studied as potential targets for inhibiting the pathogenesis of certain pathogens and have even been used as selectable markers in eukaryotic expression vectors (Degenhardt et al. 2006; Garami and Ilg 2001; Lamblin et al. 2007), very little information is available on the role of carbohydrate metabolic pathway enzymes in sporulation or secondary metabolite production in *Streptomyces*.

To determine the roles these mannose-related enzymes play, we analyzed the function of ManA in *S. coelicolor* as a continuation of our previous study on ManB. As observed in *H. pylori*, *A. fumigates*, or *B. subtilis*, deletion of *manA* was affected the morphogenesis of *S. coelicolor*, and complete loss of sporulation was observed. Our results concerning the effect of *manA* inactivation in *S. coelicolor* clearly indicate that this enzyme is vital for the production of antibiotics and cellular differentiation. Even though complementation analysis with ManA from *S. coelicolor* and *E. coli* recovered the *bld*-like phenotype, the extent of recovery varied. Partial recovery from the *bld*-like phenotype by ManA from *S. coelicolor* was observed in the complementation analysis. Compared with the substrate specificity of ManA from other organisms, ManA from *S. coelicolor* displayed low activity. In comparison to *E. coli*, the kinetic value for ManA was also very low, which could be attributed to the partial recovery from the sporulative phenotype (Roux et al. 2011).

Despite a lack of information regarding the exact target of mannosylation or how ManA affects sporulation and antibiotic production, the finding on ManA itself is quite interesting. In *S. coelicolor*, certain genes such as *ftsZ*, *ssgA*, and *ssgB* have been shown to have profound effects in determining sporulation (Flärdh et al. 2000; Flärdh and Buttner 2009; Kawamoto et al. 1997; Keijser et al. 2003). Notably, *ssgA* and *ssgB* were shown to control not only sporulation but also production of ACT. Several genes involved in the regulation of sporulation in *Streptomyces* have also been experimentally proven. The majority of other proteins encoded by similar genes function as either regulatory proteins or transcriptional factors (Xu et al. 2010). However, there has been no report of abolished sporulation and antibiotic production upon deletion of a single metabolic enzyme. Our findings suggest that mannose synthesis-related enzymes such as ManA and ManB are closely related to sporulation and antibiotic production through either control of carbon flux or direct regulation of secondary metabolism. In addition, by considering several reports on glycosylation in *Streptomyces* (Abu-Qarn et al. 2008; Ong et al. 1994; Wehmeier et al. 2009), the role of *manA* seems to be beyond just one metabolic pathway as there is evidence of several regulatory functions.

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